Karyotype Analysis of *Acacia senegal* var. *senegal* from Natural Populations in Kordofan States - Sudan*

Lubna Mohammed Babikir and Essam Ibrahim Warrag

Department of Silviculture, Faculty of Forestry, University of Khartoum, P. O. Box 13314 Shambat, Sudan

**Abstract:** The objective of this study was to find out whether there are differences in chromosome number and morphology in *Acacia senegal* in Kordofan states. Seeds were germinated and root tips were excised, prepared and examined karyotypically. The number of chromosomes was 26 (2n=2x=26) per cell but one root showed cells with 52 chromosomes, indicating the possibility of polyploidy. The chromosomes were ranked from 1 to 13 according to total length which varied from 2.80 µm to 5.41µm. According to the centromere position, the haploid set of chromosomes was classified as one median, three slightly sub-median, seven sub-median and two highly sub-median. The symmetry index and total formula were 0.51% and 43.75% respectively. The karyotypic morphology of the chromosomes can be used to detect variation within the species.

**INTRODUCTION**

*Acacia senegal* (L.) Willd. is a multi-purpose leguminous tree species of high priority in arid and semi-arid parts of the Sudan (Warrag et al. 2002). It has a wide geographical range in the Savannah belt of Africa. In the Sudan, it is distributed between latitudes 9°N and 15°N, in an area that varies in climate and soil type (Fadel Elmola 2003). The tree has wide range of environmental and socioeconomic benefits, and gum Arabic is the most important product.
The genus *Acacia* has a high ploidy level that ranges between 2x and 8x, where $2n = 2x = 26$ and $2n = 8x = 104$ (Atchison 1948; Sharma and Bhattacharyya 1958; Mehra and Bawa 1968). The chromosome number of *Acacia senegal* is $2n=2x=26$ (Bukhari 1997b). Detailed chromosomal studies of the genus *Acacia* are impaired by the numerous and small size of the chromosomes (Sharma and Bhattacharyya 1958). Bukhari (1997a) developed a method for the preparation of acacias chromosomes that emphasized the use of 75 mM potassium chloride (KCl) as a prefixing treatment of root tips to increase the spread of metaphase chromosomes.

Wide variation exists between *A. senegal* trees in gum Arabic yield and seed production. Abdel-Rahman *et al.* (2003) reported weak correlation between gum yield and morphological traits like tree height, diameter and crown. The variation in gum yield and the observed sterility of some trees (Hassan 1998) may point to variation in chromosome number and structure. Polyploidy was reported to enhance production of various secondary metabolites of plants against pathogens and pests (Kaarthik 2004). It was reported that chromosome doubling is accompanied by significantly higher production of alkaloids and terpene reaching 100% and 85%, respectively (Levin 1983). Also, the reproductive system may be influenced by chromosome doubling. Natural polyploidy was reported in acacias like *A. magium* (Turnbull *et al.* 1998), *A. dealbata* (Blakesley *et al.* 2002) and *A. mearnsii* (Beck *et al.* 2003).

The main objective of this work was to study the number and morphology of *A. senegal* chromosomes as the presence of variation in the complement may provide means of identifying markers for high yielding individual trees.
MATERIAL AND METHODS

Plant material
Seeds were collected from *A. senegal* var. *senegal* trees in Elsaata area (Elsaata Om Gamina and Elsaata Tagaano and Elsaata Bukhari), Western Kordofan State, in March 2003. Elsaata area is a major production site for gum arabic and characterized by sandy soil and annual rainfall between 300 and 450 mm. Additional seeds were collected as bulk seeds in March 2004 from Western, Southern and Northern Kordofan states.

Pilot studies were carried out to determine the suitable conditions for seed germination and root tips preparation to obtain high mitotic activities. The squash method of Dyer (1979) was modified according to Bukhari (1997a) and used to prepare root tips for examination. Seed germination at 22°C to 25°C and harvest of root tips after 3 days at 11.00 to 11.30 am gave high mitotic divisions at the first 2-3 mm from the tip of the root. Prefixing in 75Mm KCl for one hour resulted in more spread of chromosomes than in 0.1% colchicine for two hours. Colchicine gave condensed mitotically dividing cells with clustered chromosomes. This finding agrees with other reports (Sangowawa 1994; Coulaud et al. 1995)

Preparation of root tips
The permanent method of Sass (1958), with modification according to the results of the pilot studies, was used. Root tips, 2-3 mm long, were prefixed in 75mM KCl for one hour and then fixed in acetoalcohol. Dehydration was carried out through series of ethanol concentrations. The tips were then placed in 95% alcohol +1% eosin stain (stains red to make the tips observable in wax for sectioning). Clearing was done by a mixture of absolute ethanol and cedar wood oil followed by cedar wood oil and xylene and then pure xylene. The tips were then embedded in wax and sectioned by a rotary microtome, adjusted at 6µm, into long ribbons. The ribbons were cut to small sections and transferred to slides (5-6 sections from a single root tip per slide). The slides were placed over a hot plate (60°C) to melt the wax and were left to dry before staining with sefranin stain and fast green stain.
Microscopic examination
Two hundred slides with 5-6 rippons per slide (200 root tips) were examined for chromosome number. Twenty slides were taken randomly, and one cell per slide was used for measurements of the long and short arms of the chromosomes. Total chromosome length and arms ratios were calculated. The chromosomes were grouped according to arm ratios (Edwards 1979; Levan et al. 1964) and total length (Ranganath and Krishnappa 1990). Symmetry index was determined by dividing the total length of the shortest chromosome in the complement by the longest chromosome in the complement per cell (Salih 1997). Total formula percent was determined as a ratio of the sum of short arms to total length of the complement (Huziwara 1962).

RESULTS AND DISCUSSION

Chromosomes number
Metaphase chromosome number was 2n = 2x = 26 in the cells of 199 root tips, and only one root tip showed cells with 2n = 4x = 52 (Fig 1). The diploid and tetraploid numbers (2n = 2x = 26 and 2n = 4x = 52, respectively) are in agreement with those reported for a number of Acacia species, e.g., Acacia magium (Turnbull et al. 1998), Acacia dealbata (Blakesley et al. 2002), Acacia mearnsii (Beck et al. 2003) and A. senegal and Prosopis (Bukhari 1997b). Although the frequency of tetraploids in this study was low (0.5%, 1 out of 200 root tips), it points to possible presence of tetraploidy and consequently other ploidy levels in the natural population of A. senegal. Further investigation is needed to verify the presence of polyploidy and its extent in the natural populations.

Chromosomes morphology
The morphological characteristics of the haploid set of A. senegal chromosomes (n=13) are presented in Fig. 2 and Table 1. The chromosomes are arranged according to the total length in a descending order (Fig. 3). They varied in total length from 2.80 µm for the shortest to 5.41 µm for the longest. Chromosomes 1 to 5 were classified as long while the rest were medium, according to Ranganath and Krishnappa (1990). The average chromosome length of the haploid complement was 3.85 µm. These results are in contrast with earlier reports that described
Acacias chromosomes as small (Atchison 1948; Khan 1951; Sharma and Bhattacharyya 1958).

According to the long arm to short arm ratios (centromere positions), the chromosomes were grouped into three classes. Most of the chromosomes (7 out of 13) were sub-median (class 2). Class 1 comprised the median and slightly sub-median centromeres, and these were chromosomes 5, 7, 12 and 13. Class 2 with sub-median centromeres were chromosomes 1, 2, 3, 4, 6, 9 and 10. Class 3 comprised highly sub-median centromeres; these were chromosomes 8 and 11.

**Symmetry index and total formula**
The values of symmetry index and total formula were 0.51% and 43.75%, respectively. These results indicate the symmetry of the chromosome complement of *A. senegal*.

**CONCLUSIONS**
The study revealed that the chromosome number of *A. senegal* is $2n = 2x = 26$. However, there is indication of presence of the tetraploidy that deserves further studies, because of polyploids among the natural populations may explain the variation in tree production of gum arabic and seed. The morphology of the chromosomes described in this study can form the basis for future comparisons to identify chromosomal variation within the natural stands for the purpose of developing genetic markers associated with gum yield.
Table 1. Mean long arm (L), short arm (S), total chromosome length, arm ratio (L/S), centromere position (CP) and chromosome class (Ch) group of the haploid chromosome complement of *A. senegal*.

<table>
<thead>
<tr>
<th>Chromosome number</th>
<th>L.arm (μm) ±SE</th>
<th>S.arm (μm) ±SE</th>
<th>L/S ratio ±SE</th>
<th>Total length (μm) ±SE</th>
<th>CP¹</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.12 ± 0.04</td>
<td>1.36 ± 0.04</td>
<td>2.29 ± 0.04</td>
<td>5.41 ± 0.02</td>
<td>S.M.</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>2.91 ± 0.03</td>
<td>1.44 ± 0.06</td>
<td>2.02 ± 0.05</td>
<td>4.93 ± 0.02</td>
<td>S.M.</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>2.75 ± 0.07</td>
<td>1.49 ± 0.10</td>
<td>1.84 ± 0.07</td>
<td>4.59 ± 0.03</td>
<td>S.M.</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>2.61 ± 0.06</td>
<td>1.52 ± 0.10</td>
<td>1.71 ± 0.08</td>
<td>4.32 ± 0.04</td>
<td>S.M.</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>2.20 ± 0.06</td>
<td>1.15 ± 0.07</td>
<td>1.91 ± 0.04</td>
<td>4.11 ± 0.03</td>
<td>S.S.M.</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>2.33 ± 0.06</td>
<td>1.48 ± 0.10</td>
<td>1.57 ± 0.06</td>
<td>3.90 ± 0.01</td>
<td>S.M.</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>2.25 ± 0.05</td>
<td>1.50 ± 0.10</td>
<td>1.50 ± 0.06</td>
<td>3.75 ± 0.04</td>
<td>M.</td>
<td>B</td>
</tr>
<tr>
<td>8</td>
<td>2.24 ± 0.05</td>
<td>1.77 ± 0.09</td>
<td>1.26 ± 0.04</td>
<td>3.50 ± 0.04</td>
<td>H.S.M.</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>2.03 ± 0.02</td>
<td>1.70 ± 0.06</td>
<td>1.29 ± 0.03</td>
<td>3.49 ± 0.03</td>
<td>S.M.</td>
<td>B</td>
</tr>
<tr>
<td>10</td>
<td>2.01 ± 0.01</td>
<td>1.70 ± 0.05</td>
<td>1.17 ± 0.03</td>
<td>3.72 ± 0.03</td>
<td>S.M.</td>
<td>B</td>
</tr>
<tr>
<td>11</td>
<td>1.94 ± 0.04</td>
<td>1.79 ± 1.08</td>
<td>1.08 ± 0.04</td>
<td>3.02 ± 0.01</td>
<td>H.S.M.</td>
<td>B</td>
</tr>
<tr>
<td>12</td>
<td>1.59 ± 0.03</td>
<td>1.11 ± 0.03</td>
<td>1.44 ± 0.03</td>
<td>3.03 ± 0.00</td>
<td>S.S.M.</td>
<td>B</td>
</tr>
<tr>
<td>13</td>
<td>1.56 ± 0.04</td>
<td>1.25 ± 0.05</td>
<td>1.24 ± 0.03</td>
<td>2.80 ± 0.02</td>
<td>S.S.M.</td>
<td>B</td>
</tr>
</tbody>
</table>

¹ CP =M., S.S.M; S.M. and H.S.M. indicate median (L/S= 1.00–1.10), slightly sub-median (L/S= 1.11–130), sub-median (L/S= 1.31–1.7) and highly sub-median (L/S>1.71) centromere position, respectively (Edward 1979).

² Ch class= According to Ranganath and Krishnappa (1990): A= Long chromosome> 4 um, B = medium chromosome 4.0-2.5 um.
Fig. 1. A tetraploid cell (2n = 4x = 52) of Acacia senegal

Fig. 2. Chromosome complement of Acacia senegal (2n=2x =26)
Fig. 3. Ideogram of *Acacia senegal* chromosome complement
REFERENCES


تحليل النمط الصبغي لأشجار الهشاب في عشائر طبيعية بولايات كردفان (السودان)

ليلى محمد بابكر و عماد الدين إبراهيم و راق
قسم فلالة الغابات، كلية الغابات - جامعة الخرطوم
شمال-السودان

موجز البحث: هدفت هذه الدراسة إلى تقسيم وجود تباين من حيث الشكل والعدد في صبغيات أشجار الهشاب في ولايات كردفان. تم إنبات البذور تحت ظروف ملائمة للحصول على خلايا في طور الانقسام الميتوزي، وقطع القمم النامية للجذور وجعلت وفحصت لدراسة النمط الصبغي. كان عدد الصبغيات في 199 من خلايا القمم النامية 26 (2x=2n) = 52 صبغياً مما يشير إلى احتمال وجود نباتات رباعية الطاقم الصبغي. قسمت الصبغيات حسب أطوالها من 1 إلى 13 وتراوحت الأطوال بين 2.8 مايకرون و 5.14 مايکرون. بناءً على موقع المستروم صنفت صبغيات المجموعة الأحادية إلى 1 وسطي و 3 نوعاً ما شبه وسطي و 7 شبه وسطي و 2 عالي شبه وسطي. وبلغ معامل التماثل والصبغة الكلية 51.0% و 43.75% على التوالي. ويمكن استخدام النمط الصبغي في دراسة التباين في أشجار الهشاب.